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Serum levels of pregnancy associated α_2-glycoprotein (α_2-PAG) during pregnancy in autoimmune thyroid disease: relationship to disease activity

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SUMMARY

Serum concentrations of pregnancy associated α_2-glycoprotein (α_2-PAG) were measured serially by an enzyme linked immunosorbent assay during 24 pregnancies in 18 patients with Graves' disease and four with Hashimoto's thyroiditis. During each trimester, α_2-PAG levels were significantly higher than in normal pregnant controls, matched for week of gestation. Patients showing remission of disease activity had progressively higher α_2-PAG levels throughout pregnancy than those with active disease. The data support the idea that α_2-PAG may play an important role in inducing and maintaining the clinical remissions observed in some women with autoimmune thyroid disease during pregnancy.

Keywords Graves' disease Hashimoto's thyroiditis pregnancy pregnancy associated α_2-glycoprotein

INTRODUCTION

Women suffering from autoimmune disorders such as rheumatoid arthritis, Graves' disease and Hashimoto's thyroiditis often show amelioration of disease activity during pregnancy, with a tendency to relapse in the post-partum period. In the context of autoimmune thyroid disease, Amino *et al.* (1978) showed that titres of both anti-thyroglobulin and anti-thyroid microsomal antibodies decreased as pregnancy progressed, with a transient increase in antibody levels following delivery. More recently, Hardisty & Munro (1983) reported a decline in activity of long acting thyroid stimulator protector as pregnancy progressed in patients with Graves' disease. The basic mechanism(s) inducing such changes is unknown.

Numerous factors have been proposed as possible mediators in the suppression of an anti-fetal allograft response, including gestational hormones, blocking antibodies and pregnancy associated proteins. Of the latter, there is experimental evidence indicating that the high molecular weight glycoprotein, pregnancy associated α_2-glycoprotein (α_2-PAG, synonyms: SP3, PZ protein), can exert immunosuppressive effects (Damber *et al.*, 1975; Stimson, 1976; Svendsen *et al.*, 1978). This protein is detectable in very small amounts in all human sera and concentrations usually rise dramatically during pregnancy as well as following oestrogen administration (for references see Horne, Thomson & Armstrong, 1982).

In rheumatoid arthritis, it has been postulated that in some women, the lack of remission during

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pregnancy may be due to their failure to produce high concentrations of α_2-PAG (Persellin, 1977). This hypothesis is supported by the recent finding of Unger *et al.* (1983) that patients with rheumatoid arthritis whose disease activity diminishes during pregnancy have significantly higher α_2-PAG levels than those whose disease activity increases or remains unchanged throughout gestation. These observations have prompted us to conduct a longitudinal study of serum α_2-PAG levels in 22 patients with autoimmune thyroid disease during pregnancy. Our aim was to determine whether any relationship could be found between disease activity and α_2-PAG concentrations.

MATERIALS AND METHODS

Patients. Twenty-two patients (mean age 26 years, range 19–35) with autoimmune thyroid disease and attending the Aberdeen Thyroid Clinic were studied before and during a total of 24 pregnancies. Eighteen patients had Graves' disease and four had Hashimoto's thyroiditis, one patient from each group having been investigated during two successive pregnancies. The criteria for the diagnosis of Graves' disease included the presence of a diffuse goitre (with or without ophthalmopathy) and circulating thyroid autoantibodies (thyroglobulin and/or microsomal) as well as clinical evidence and laboratory confirmation of hyperthyroidism, either prior to or during pregnancy. The diagnosis of Hashimoto's thyroiditis was based on the presence of goitre (associated with hypothyroidism in two patients) and high titres of thyroid autoantibodies.

Based on serial clinical and laboratory findings, but without prior knowledge of their serum α_2-PAG concentrations, 12 pregnancies (nine Graves' disease and three Hashimoto's thyroiditis) were associated with remission of disease (group 1) and the other 12 (10 Graves' disease and two Hashimoto's thyroiditis) with active disease (group 2). In group 1, the patients with Graves' disease were hyperthyroid prior to conception and were treated with carbimazole, which was stopped either just before pregnancy ($n=4$) or during the first trimester ($n=5$); thereafter, these patients remained clinically and biochemically euthyroid. In contrast, the patients with Graves' disease in group 2 were thyrotoxic during pregnancy and required carbimazole therapy well into the post-partum period. Hashimoto's thyroiditis was judged to be in remission by the maintenance of normal thyroid status without treatment and a marked fall in the titres of thyroid autoantibodies during pregnancy; active disease was associated with persistently high titres of thyroid autoantibodies throughout pregnancy while on thyroxine replacement therapy. The patients in groups 1 and 2 were comparable with regard to age and parity (Table 1).

Sera obtained from the patients were stored at $-20°C$ and assayed for α_2-PAG simultaneously with sera from 86 normal pregnant women matched for week of gestation.

Estimation of thyroid autoantibodies. Anti-thyroglobulin and anti-thyroid microsomal antibodies were measured by the tanned red cell haemagglutination technique, using commercially available test kits ('Thymune T' and 'Thymune M', Wellcome Reagents Ltd.) Serial dilutions of the

Table 1. Classification of pregnancies in patients with autoimmune thyroid disease

Patients ($n=22$)		Group 1 (remission)	Group 2 (active disease)
Pregnancies	Total	12	12
	Graves' disease	9	10
	Hashimoto's thyroiditis	3	2
Age	mean	26·3 years	26·9 years
	range	21–35	19–31
Parity	0+0	4	3
	1+0	6	6
	2+0	2	3

initial 1:5 dilutions of sera were performed in U shaped microtitre plates (Titertek) using a semi-automatic multiple diluter. Titres of 1:100 (anti-thyroid microsomal antibodies) or 1:10 (anti-thyroglobulin) were considered to be positive.

Immunoassay of α_2-PAG. The concentration of α_2-PAG in serum samples was determined using an enzyme immunoassay (ELISA) recently developed in the Department of Pathology. Briefly, MicroELISA trays (Dynatech) were coated overnight at room temperature with sheep anti-human α_2-PAG (Seward) at a dilution of 1 in 1,000 (= 25 mg protein/l) in 50 mmol/l carbonate/bicarbonate buffer, pH 9·6. The trays were washed using 0·1% (vol./vol.) Tween-20 in phosphate-buffered saline (PBS = 0·15 mol/l NaCl, 10 mmol/l sodium phosphate buffer, pH 7·2) and then incubated in turn with the following. 1st incubation appropriate dilutions of serum samples. 2nd incubation rabbit anti-human α_2-PAG (IgG fraction) at a dilution of 1 in 2,500 (= 10 mg protein/l). 3rd incubation goat anti-rabbit IgG–peroxidase conjugate (Sigma) at a dilution of 1 in 7,000 (stock solution contains approximately 6–8 purpurogallin units/ml). The plates were thoroughly washed prior to the addition of each reagent and incubations were carried out for 90 min at 25°C using volumes of 200 µl/well. The diluent used in each case was PBS containing 1% (wt/vol.) bovine serum albumin.

Following the third incubation, 200 µl/well of 0·15 mol/l citrate/phosphate buffer, pH 5·0 containing 10 mM *o*-phenylenediamine and 0·03% (vol./vol.) H_2O_2 was added and the peroxidase reaction left to proceed for 30 min in the dark. The reaction was stopped by the addition of 50 µl of 4 M H_2SO_4 and the intensity of the final colour reaction measured spectrophotometrically at 486 nm using a Kontron SLT210 Automatic Analyser. A standard curve of samples containing known amounts of α_2-PAG (3–1,000 µg/l) was prepared from standardized pooled pregnancy sera (Behringwerke AG) and included in each assay. The concentration of α_2-PAG in each test sample was then determined directly from this curve.

Statistics. Results are presented as means ± 1 s.d. The significance of differences was determined using the Student's *t*-test for unpaired samples. Since in common with many circulating molecules, α_2-PAG levels are distributed asymetrically about the mean, comparisons are made following logarithmic transformation of these values (Thomson *et al.*, 1979b; Folkersen *et al.*, 1981).

RESULTS

Serial serum α_2-PAG concentrations in the 22 patients with autoimmune thyroid disease are shown in Fig. 1. Levels rose steadily from 71·4 ± 49·0 mg/l at 2–10 weeks gestation to a peak value of 919 ± 382 mg/l at weeks 26–30 gestation. Thereafter, α_2-PAG concentrations tended to decrease, although this was not particularly evident from the mean value for the period 31 weeks to term

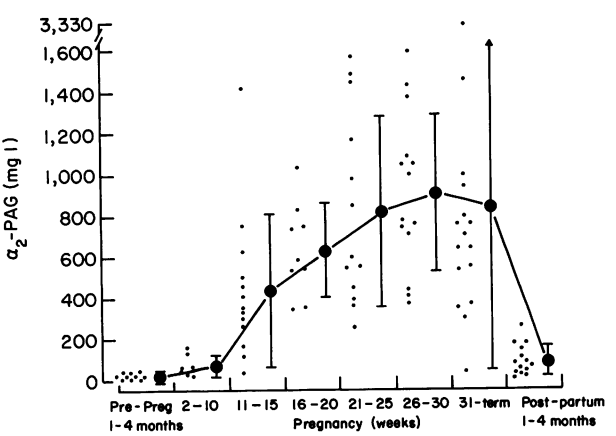

Fig. 1. Serum α_2-PAG concentrations (arithmetic mean ± 1 s.d.) during pregnancy in patients with autoimmune thyroid disease.

(851 ± 794 mg/l). This value was however falsely accentuated by an isolated exceedingly high α_2-PAG concentration (3,330 mg/l) in one patient: exclusion of this value would bring the mean to 661 ± 362 mg/l. Serum α_2-PAG levels measured in the post-partum period were within the normal range for non-pregnant women.

Serial concentrations of anti-thyroid microsomal antibodies are shown in Fig. 2. There was a four-fold decrease in the mean titre of antibodies between early pregnancy and the period corresponding to maximal α_2-PAG concentrations (week 26–term). Indeed, 45% of patients were negative for anti-thyroid microsomal antibodies by the third trimester. However, no significant correlation between serum α_2-PAG levels and titres of anti-thyroid microsomal antibodies was established. The number of patients with positive tests for anti-thyroglobulin antibody was too small to allow any conclusions to be drawn.

Fig. 3 shows the mean α_2-PAG levels during first, second and third trimester for all patients with autoimmune thyroid disease, those in the 'remission' and 'active disease' categories and normal pregnant controls matched for week of gestation. For each trimester, not more than 1 sample per patient was measured and samples in all categories were randomly distributed with respect to week of gestation.

When compared with normal pregnant women, α_2-PAG levels in patients with autoimmune thyroid disease were significantly higher for each trimester. There was no significant difference between patients with Graves' disease and those with Hashimoto's thyroiditis. However, patients in remission (group 1) showed higher α_2-PAG levels than those with active disease (group 2), the difference increasing as pregnancy progressed. Indeed, in the third trimester the difference was greater than two-fold (remission 1,320 mg/l; active disease 580 mg/l; $P < 0{\cdot}01$). Both active disease and remission groups had significantly higher α_2-PAG levels than normal pregnant controls during the second trimester, but the difference was significant only for the remission group during the third trimester.

DISCUSSION

Our data show that throughout gestation, serum α_2-PAG concentrations are significantly higher in patients with autoimmune thyroid disease than in normal pregnant women, values of the latter being similar to those reported previously in normal primigravidae (Bohn & Winckler, 1976). It is also evident that, as pregnancy progresses, this difference becomes greater. Moreover, we have demonstrated that among patients with autoimmune thyroid disease, remission of disease activity during gestation is associated with higher levels of α_2-PAG than those found in pregnant patients with active disease.

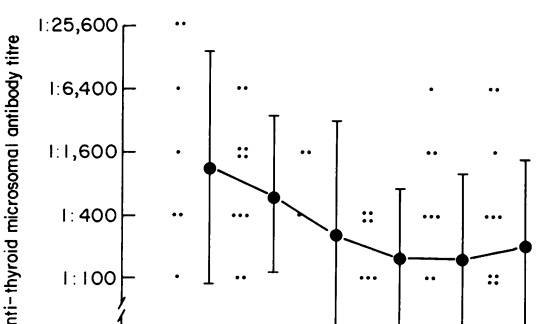

Fig. 2. Anti-thyroid microsomal antibody titres (arithmetic mean ± 1 s.d.) during pregnancy in patients with autoimmune thyroid disease.

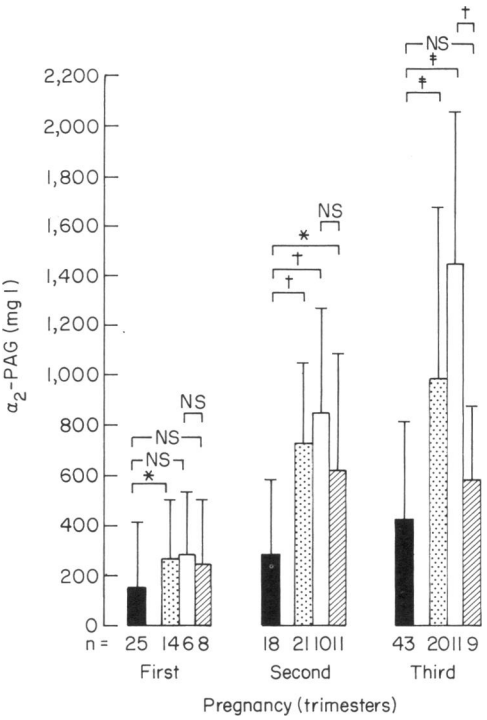

Fig. 3. Mean serum α_2-PAG concentrations (arithmetic mean $\pm$ 1 s.d.) during each trimester in patients with autoimmune thyroid disease and in normal pregnant controls matched for week of gestation. n = number of patients. Symbols indicate significance of differences between groups following log transformation of data: *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$; NS = not significant. ■ = normal controls; ▨ = all patients; □ = remission; ■ = active disease.

It is unclear why serum α_2-PAG levels should be higher in women whose pregnancies are complicated by autoimmune thyroid disease. However, Straube *et al.* (1980) have reported that patients with type I insulin-dependent diabetes mellitus also have elevated levels of α_2-PAG during the third trimester of pregnancy, especially where there is concomitant rhesus D antigen incompatibility. These authors have excluded the possibility of excess oestrogen production being the causal factor in the elevation of α_2-PAG levels above those in normal pregnancy.

Serum α_2-PAG levels have previously been examined in non-pregnant patients with a variety of autoimmune disorders and raised levels of the glycoprotein have been reported in rheumatoid arthritis (Horne *et al.*, 1979; Kasukawa *et al.*, 1979) and in the putative autoimmune disorders psoriasis (Beckman *et al.*, 1979) and Behçet's syndrome (Thomson *et al.*, 1981). On the other hand, in a series of non-pregnant patients with thyrotoxic Graves' disease, Horne *et al.* (1978) found decreased levels of α_2-PAG compared to age and sex matched controls ($74{\cdot}4 \pm 65{\cdot}9$ mg/l *vs* $116{\cdot}9 \pm 709$ mg/l; $P < 0{\cdot}05$). However, serial measurements following treatment were not undertaken in the latter study to explore the possibility that the hyperthyroid state *per se* may have had an influence on the α_2-PAG levels. Our data suggest this is unlikely since our patients with active Graves' disease continued to show lower α_2-PAG concentrations compared to patients in remission despite correction of the hyperthyroidism with carbimazole treatment. This is further supported by our findings in patients with Hashimoto's thyroiditis who remained euthyroid throughout pregnancy; observations in this same group would also tend to exclude a direct effect of carbimazole itself on α_2-PAG levels. Moreover, within the group of patients in remission of Graves' disease, those on no drug treatment had similar α_2-PAG concentrations as patients still on carbimazole during the first trimester of pregnancy.

In addition to Graves' disease, Horne *et al.* (1978) also found significant reductions in α_2-PAG levels in patients with atrophic gastritis associated with intrinsic factor antibodies. The authors speculated that in the latter instance the low α_2-PAG levels might be related to leucopenia associated with megaloblastic anaemia, since there is evidence that α_2-PAG is synthesized by blood leucocytes. In the presence of oestrogen, peripheral blood mononuclear cells secrete α_2-PAG, which can be detected on the surface membrane of these cells (Stimson & Blackstock, 1975; Stimson, 1977; Thomson *et al.*, 1979b). It is also found within plasma cells in lymphoid tissue (Thomson & Horne, 1980) and in sites of inflammation, such as inflamed gut mucosa (Horne *et al.*, 1983).

Our findings are in keeping with those of Unger *et al.* (1983), who detected higher levels of α_2-PAG in those pregnant rheumatoid arthritis patients who showed concurrent remission of disease activity. These authors were unable to find an association between the concentrations of α_2-PAG and IgM rheumatoid factor, and likewise we have not observed any correlation between glycoprotein concentrations and thyroid autoantibody levels. However, there is increasing evidence that cell-mediated as well as humoral immunity may participate in the pathogenesis of autoimmune thyroid disease (Volpé, 1981). It is therefore relevant that the reported immunosuppressive properties of α_2-PAG, derived from studies of antigen-induced leucocyte migration inhibition (Stimson, 1976), mixed lymphocyte reactivity (Damber *et al.*, 1975; Stimson, 1976, 1980) and allograft rejection (Svendsen *et al.*, 1978) are mediated against T cells. Although a number of possible mechanisms may account for depression of maternal immune reactivity (reviewed in Wegmann & Gill, 1983), our results indicate that α_2-PAG, a maternally derived glycoprotein may play an important role in the induction and/or maintainance of remission observed in some patients with autoimmune thyroid disease during pregnancy.

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